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LATHAM & WATKINS LLP

File No. 024150-0025

Contain NO CBI

July 28, 2003

VIA MESSENGER

Ed Gross
Office of Pollution Prevention & Toxic Substances
Room 6104
1200 Pennsylvania Avenue
Washington, DC 20460

Re: 3M Company TSCA 8(e) Submissions

Dear Mr. Gross:

Pursuant to your request, enclosed please find complete copies of the following studies 3M Company submitted to the Section 8(e) Coordinator on May 23, 2003:

1. Study No. 3: Subchronic 90-Day Oral Toxicity with T-6524 by Daily Gavage in the Rat Followed by a 28-Day Recovery Period

2. Study No. 8: Acute Oral Toxicity Study in Rats (Exp. No. 930321) (Test Article: 501149)

3. Study No. 17: Dermal Sensitization Study of T-7280 in Guinea Pigs – Closed Patch Technique (with Protocol TP 2008 attached)

4. Study No. 25: One Generation Reproduction Study of PFOS – Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats

I would greatly appreciate it if you could replace the inadvertently incomplete versions of these studies submitted on May 23, 2003 with the enclosed complete versions of the studies.

Please note that these studies do not contain confidential business information.

If you have any questions regarding the enclosed documents, please do not hesitate to call me at (202) 637-2144.

I apologize for any inconvenience.

Sincerely,

Debra Bhaumik

Debra Bhaumik
Paralegal

cc: Julia A. Hatcher, Esq.

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3M General Offices

3M Center
St. Paul, MN 55144-1000
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May 23, 2003

mL #266985

3M **By Hand Delivery**

Document Processing Center (7407)
Office of Pollution, Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460
Attention: Section 8(e) Coordinator

Re: **TSCA Section 8(e) Submissions**

Dear Sir/Madam:

3M Company ("3M") requests that EPA place the attached studies in the TSCA Section 8(e) docket. We have included an index for these studies identifying the study title, test substance and CAS number. A CBI version of this index and the studies also is being submitted today pursuant to EPA procedures.

3M has concluded that data in these studies may not be, strictly speaking, "corroborative" of previously reported or published information as defined in EPA's reporting guidance or otherwise potentially may warrant 8(e) submission based on EPA's reporting guidance.

3M appreciates EPA's attention to this matter. Please contact the undersigned if you have any questions or require further information regarding this submission.

Very truly yours,

Dr. Katherine E. Reed (GH)

Dr. Katherine E. Reed, Ph.D
Executive Director
3M Environmental Technology
And Safety Services
(651) 778-4331
kereed@mmm.com

SUBMISSION BY 3M COMPANY ON MAY 23, 2003

1.	Exploratory 28-Day Oral Toxicity Study with T-7250, T-7251, T-7252, T-7253, T-7254, and T-7255 by Daily Gavage in the Rat Followed by a 14/28-Day Recovery Period (NOTOX Project 264656)	Separate studies for each chemical: [CBI removed]; Hexanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,6 - Tridecafluoro-n-(2-Hydroxyethyl)-N-Methyl - 100%; 1-Butanesulfonamide, 1,1,2,2,3,3,4,4,4-Nonafluoro-N-n(2-Hydroxyethyl)-N-Methyl - 100%	[CBI removed]; 68555-75-9; 34454-97-2
2.	Exploratory 28-Day Oral Toxicity Study with T-7125, T-7126, T-7127, T-7128, and T-7129 by Daily Gavage in the Rat Followed by a 14/28-Day Recovery Period (NOTOX Project 256679)	Cyclohexanesulfonic acid, decafluoro(pentafluormethyl)-, potassium salt (CAS No. 67584-42-3) - 66-70%; Cyclohexanesulfonic acid, decafluoro(trifuloromethyl)-, potassium salt (CAS No. 68156-07-0) - 18-22%; Cyclohexanesulfonic acid, nonafluorobis(trifluoromethyl)-, potassium salt (CAS No. 68156-01-4) - 9-13%; Cyclohexanesulfonic acid, undecafluoro-, potassium salt (CAS No. 3107-18-4) - 1-3%	67584-42-3; 68156-07-0; 68156-01-4; 3107-18-4
3.	Subchronic 90-Day Oral Toxicity with T-6524 by Daily Gavage in the Rat Followed by a 28-Day Recovery Period	65% Sulfonamides, C4-8-alkane, perfluoro, N-(3-(dimethyloxidoamino)propyl), potassium CAS#179005-06-2; 20% Amine oxide C8F17SO2NH(->O)CH2CH2CH2N(CH3)2; 15% C3-C7 K-salts of amine oxides CNF2N+1SO2N-)(+K)(->O)CH2CH2CH2N(CH3)2	179005-06-2
4.	A Study for Effects on Embryofoetal Development of the Rat (Inhalation Administration)	[CBI removed]	[CBI removed]
5.	Evaluation of the Ability of T-5870 to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (with Independent Repeat)	2-ethoxy ethyl acrylate	106-74-1
6.	Chromosomal Aberration Test of T-6695 Using Cultured Mammalian Cells	[CBI removed]	[CBI removed]
7.	Acute Oral Toxicity Study in Rats (Exp. No. 920584) (Test Article: Intermedio 1249)	2-methyl-2-butanone-(4-sulfonamidophenyl)-hydrazone; Molecular Formula: C11H17N3O2S	Unknown
8.	Acute Oral Toxicity Study in Rats (Exp. No. 930321) (Test Article: 501149)	3H-pyrazol-3-0ne, 2-(4-aminophenyl), 4-dihydro-5-(1-pyrrolidinyl)	30707-77-8
9.	Skin Corrosivity Study of T-5799 in Rabbits (DOT/UN Regulations)	1-Octanesulfonyl Fluoride - 87.5%, Other Alkyl Sulfonyl Fluorides and Acidic Impurities - 11%, Water - 5.4%, Octanesulfonyl Chloride - 1.4%	40630-63-5; Unknown; 7732-18-5; 7795-95-1
10.	Skin Corrosivity Study of T-5800 in Rabbits (DOT/UN Regulations)	1-Octanesulfonyl Fluoride - 87.5%, Other Alkyl Sulfonyl Fluorides and Acidic Impurities - 11%, Water - 5.4%, Octanesulfonyl Chloride - 1.4%.	40630-63-5; Unknown; 7732-18-5; 7795-95-1
11.	Primary Dermal Irritation/Corrosion Study of T-5635 in Rabbits (OECD Guidelines)	[CBI removed]	[CBI removed]
12.	Primary Dermal Irritation/Corrosion Study of T-5897 in Rabbits (OECD Guidelines)	Isophthaloylbis (2-methylarziridine) - 97%, Toluene - 2%, Xylene - 0.5%.	7652-64-4; 108-88-3; 1330-20-7
13.	Skin Corrosivity Study of T-7030.1 in Rabbits (with Protocol TP4206 attached)	[CBI removed]	[CBI removed]
14.	Dermal Sensitization Study of T-5474 in Guinea Pigs - Maximization Test (EPA Guidelines)	Water (CAS No. 7732-18-5) - 68.4%; Dodecylbenzenesulfonic Acid (CAS No. 27176-87-0) - 17.5%; Polymethacrylate (CAS No. 25087-26-7) - 11.76%; Sodium Hydroxide (CAS No. 1310-73-2) - 2.3%; Unknown - 0.040%	7732-18-5; 27176-87-0; 25087-26-7; 1310-73-2

SUBMISSION BY 3M COMPANY ON MAY 23, 2003

		Test Substance	
15	Dermal Sensitization Study of T-5894 in Guinea Pigs - Maximization Test (EC Guidelines) (with Protocol TP6164E attached)	[CBI removed]	[CBI removed]
16.	Dermal Sensitization Study of T-6006 in Guinea Pigs - Closed Patch Technique (EPA Guidelines)	Dimethyltetradecylamine Oxide - 55%, Oleamidopropyldimethylamine - 18%, 1-Methoxy -2-Propanol – 5%, Citronellol – 5%, Polyethylene Glycol - < 3%, Alpha - (Carboxymethyl) - Omega - (Dodecyloxyl) Poly (Oxyethylene) Sodium Salt - ~3%, Trialkyl Amine Oxide - 2%, Isopropyl Alcohol - 2%, Fragrance Sozio SZ 5467 - 2%, Water - 1%, Acetic Acid – 1%, Miscellaneous ingredients at less than 1%	3332-27-2; 109-28-4; 107-98-2; 106-22-9; 25322-68-3; 33939-64-9; 7128-91-8; 67-63-0; Unknown; 7732-18-5; 64-19-7
17.	Dermal Sensitization Study of T-7280 in Guinea Pigs - Closed Patch Technique (with Protocol TP2008 attached)	[CBI removed]	[CBI removed]
18.	Acute Oral Toxicity Study of T-6735 in Rats (OECD Guidelines) (with Protocol TP2069 attached)	4,6-dibromo-2-isopropyl phenol	Unknown
19.	Acute Toxicity to Daphnia Magna	[CBI removed]	[CBI removed]
20.	Evaluation of the Mutagenic Activity of T-5870 in an In Vitro Mammalian Cell Gene Mutation Test with L5178Y Mouse Lymphoma Cells (with Independent Repeat)	2-ethoxy ethyl acrylate	106-74-1
21.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 920364) (Test Article: 586442-50055)	HP=Benzothiazolium (9CI); SB=3-ethyl-2-((3-(3-(3-ethyl-2(3H)-benzothiazolylidene)-1-propenyl)-5,5-dimethyl-2-cyclohexen-1-ylidene)methyl)-6-methoxy-5-methyl-; NM=Iodide; Molecular Formula: C32H37N2OS2.I	87699-86-3
22.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 940151) (Test Article: 580066)	Thiazolium, 3-ethyl-2-[3-(3-ethyl-2-thiazolidinylidene)-1-propenyl]-4,5-dihydro-,iodide; Molecular Formula: C13H21N2S2.I	3065-71-2
23.	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 930529) (Test Article: 1268)	3-ethoxy-carbonyl-methyl-4-etoxy-methylidene-rhodanine; Molecular Formula: C10H13NO4S2	Unknown
24	Acute Eye Irritation Study in New Zealand White Rabbits (Exp. No. 920582) (Test Article: 1248)	C6H10ClN3O2S	Unknown
25.	One Generation Reproduction Study of PFOS - Mevalonic Acid/Cholesterol Challenge and NOEL Investigation in Rats	Perfluorooctane Sulfonic Acid Potassium Salt	2795-39-3
26	Augmented acute (4-hour) inhalation toxicity study with T-6905 in rats	2% solids of fluorochemical fatty acid ester in water	306974-63-0

Final Report

Dermal Sensitization Study of T-7280 in Guinea Pigs - Closed Patch Technique

PREPARED FOR:
3M

COVANCE STUDY NUMBER:
90703666

ISSUE DATE:
January 27, 2000

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545
Deliveries: 3301 Kinsman Blvd., Madison, WI 53704
Tel: 608/241-4471 Fax: 608/241-7227

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

3M
St. Paul, MN

FINAL REPORT

Study Title:

Dermal Sensitization Study of T-7280 in Guinea Pigs – Closed Patch Technique (EPA/OECD Guidelines)

Author:

Steven M. Glaza

Study Completion Date:

January 27, 2000

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Project Identification:

Covance 90703666

COMPLIANCE STATEMENT

Dermal Sensitization Study of T-7280 in Guinea Pigs –
Closed Patch Technique
(EPA/OECD Guidelines)

This study was conducted in accordance with the following Good Laboratory Practice Standards with the exception that analysis of the test and component material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17

Steven M. Glaza
Study Director
Toxicology
Covance Laboratories Inc.

1-27-00
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and Study Director Management
From	To	Phase	
09/23/99	09/23/99	Test Article Preparation	09/23/99
12/28/99	12/28/99	Data Review	12/28/99
12/28/99	12/28/99	Report Review	12/28/99

Susan K. Swatsh

Representative, Quality Assurance Unit

1-27-00

Date

STUDY IDENTIFICATION

Dermal Sensitization Study of T-7280 in Guinea Pigs – Closed Patch Technique (EPA/OECD Guidelines)

Test Material	T-7280
Sponsor	3M Corporate Toxicology 3M Medical Department 3M Center, Building 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor's Representative	John L. Butenhoff, PhD, DABT 3M Telephone No.: 651.733.1962 Facsimile No.: 651.733.1773
3M Study Monitor	Ken Nakatani, MS, DABT 3M Telephone No.: 651.736.7428 Facsimile No.: 651.733.1773
Study Director	Steven M. Glaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Testing Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	September 10, 1999
Experimental Start Date	September 13, 1999 (initiation of treatment)
Experimental Termination Date	November 22, 1999

KEY PERSONNEL

Toxicology

Steven M. Glaza
Study Director

Rose M. Bridge
Administrative Supervisor

Toxicology Operations

Jeffrey B. Hicks
In-life Supervisor

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Associate Director

CONTENTS

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of a test material in guinea pigs.[1,2] The closed patch study methodology was utilized as a previous attempt to conduct a Magnusson-Kligman maximization test was unsuccessful due to the test material's insolubility in various vehicles.

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office for Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as T-7280 and described as a viscous, tan liquid.

Purity and Stability

The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused test material will be discarded after issuance of the final report according to Covance SOP and the Sponsor's instructions.

Test Material Component Materials

The Sponsor provided the test material component material (Curing Agent 5974P), described as an opaque white crystalline solid, that was used in the rechallenge phase.

This material was stored at room temperature. A reserve sample of the material was taken and will be retained in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused material will be discarded after issuance of the final report according to Covance SOP.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc. as follows:

Treatment Group	Date Received	Location
Irritation Screening Studies		
Initial	August 24, 1999	Kingston, New York
Second	September 7, 1999	St. Constant, Canada
Third	October 5, 1999	Kingston, New York
Definitive Study		
Test Group and Naive Control	September 7, 1999	St. Constant, Canada
Additional Naive Control	October 11 and 18, 1999	Portage, Michigan

Housing

After receipt, the animals were acclimated for a period of at least 5 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18 to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to certified guinea pig diet (#5026, Purina Mills, Inc.) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically

analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Forty-seven healthy, acclimated male albino guinea pigs, weighing from 403 to 606 g and approximately 6 to 10 weeks of age, were identified by animal number and corresponding ear tag and divided into the following treatment groups:

Identification	Number of Animals
Irritation Screening Studies	
Initial irritation screening (T-7280)	4
Second irritation screening (T-7280)	4
Third irritation screening [a] (Curing Agent 5974P)	4
Definitive Study	
Test (T-7280)	20
Naive Control	10
Additional Naive Control	5

a Screening of component material used in the rechallenge phase.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Irritation Screening Studies

Initially, an irritation screening study using four animals was conducted to determine the irritation threshold of the test material. The test material (T-7280) was administered undiluted and at concentrations of 25, 50, and 75% w/v in 80% v/v ethanol in distilled water with each animal receiving all four concentrations of the test material. The appropriate test material concentrations, in the amount of 0.4 mL, were applied to

adhesive patches (Hill Top Chamber®, 25-mm diameter). The patches were then placed on four shaved sites (two on the right side and two on the left side) on each animal, covered with an overlapping strip of dental dam, and overwrapped with Elastoplast® tape. The patches remained in place for 6 hours after which they were removed. Any residual material was then removed from the application sites using liquid Ivory® soap mixed with water, rinsed with water, and dried with disposable paper towels. The application sites were observed for dermal reactions at 24 and 48 hours after test material application.

Based on the irritation observed after the first induction dose to the undiluted test material, a second irritation screening study was conducted. Four additional animals were treated with the test material (T-7280) undiluted and at concentrations of 25, 50, and 75% w/v in 80% v/v ethanol in distilled water in the same manner as the first irritation screening study.

Prior to the conduct of the rechallenge phase in the definitive study, an additional irritation screening study was conducted to determine the irritation threshold of the component material. The four animals in the third irritation screening study were treated with Curing Agent 5974P at concentrations of 10, 25, 50, and 75% w/v in distilled water also in the same manner as the first irritation screening study.

Definitive Study

Based on the results of the initial irritation screening studies, the test material was administered undiluted for each application in the induction phase and as a 75% w/v mixture in 80% v/v in ethanol in distilled water for the initial challenge application. All test material mixtures used in the irritation screening or definitive phases of the study were stored at room temperature until administered.

Induction Phase. On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The undiluted test material was applied to each animal in the test group by placing 0.4 mL on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual test material was then removed from the application sites using liquid Ivory® soap mixed with water, rinsed with water, and

dried with disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications. The naive control animals were not treated during this phase of the study.

Initial Challenge Phase. Two weeks following the administration of the third induction dose, a challenge dose of 0.4 mL of a 75% w/v mixture of the test material in 80% v/v ethanol in distilled water was administered along the dorsal anterior right quadrant of the test group animals in the same manner as during the induction phase of the study. At this time the 10 naive (previously untreated) control animals were also treated in the same manner with a challenge application of the test material.

Rechallenge Phase. To further examine the sensitizing potential of the test material, a rechallenge dose was conducted on Day 57 with a test material component. A new naive site was selected on each respective animal in the test group. In addition, five new naive control animals were selected and a site prepared on these animals. Each animal in the test and additional naive control groups received a 0.4 mL dose of a 10% w/v mixture of the test material component Curing Agent 5974P in distilled water.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the irritation screening and challenge applications, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour observation occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[3] scoring scale at 24 and 48 hours following the irritation screening, induction, and challenge applications.

Clinical observations were conducted daily throughout the study. Body weights on the irritation screening animals were determined only on the day of treatment. Body weights

on the definitive study animals were determined before the initial test material administration and at termination of the respective in-life phase.

Termination

At termination of the respective in-life phase for each group, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals may indicate evidence of sensitization, provided grades of less than 1.0 are seen in the naive control animals.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Irritation Screening Study

Individual body weights and dermal reactions for the irritation screening studies are in Tables 1, 2, and 3. Only a very faint erythema reaction was observed in one animal to the undiluted test material applied during the initial irritation screening phase. No other dermal irritation was observed. It was determined that undiluted T-7280 would be the appropriate concentration for the induction phase.

In the second irritation screening, very faint to faint erythema reactions were observed in all four animals at the sites treated with the undiluted test material, and very faint erythema reactions were observed in two of the four animals to the 25, 50, and 75% w/v

concentrations of the test material in 80% v/v ethanol in distilled water. Pinpoint areas of subcutaneous hemorrhaging were also observed. Based on these results, a 75% w/v mixture of the test material in 80% v/v ethanol in distilled water was used for the initial challenge application.

In the irritation screening of Curing Agent 5974P, a very faint erythema reaction was observed in one animal to the 10% w/v concentration in distilled water, very faint to faint erythema reactions were observed in three animals to the 25% w/v concentration and all four animals to the 50% w/v concentration in distilled water. Faint to moderate erythema reactions were observed in all four animals to the 75% w/v concentration of Curing Agent 5974P in distilled water. It was determined that the 10% w/v mixture in distilled water would be the appropriate concentration for the rechallenge phase.

All animals appeared normal during this phase of the study.

Definitive Study

Clinical Observations and Body Weights. Individual body weights are in Table 4 and individual clinical signs are in Table 5. All animals in all groups appeared normal throughout the study with the exception of one animal in the test group which appeared dehydrated and was given a water bottle on Day 15. This animal also exhibited a thin appearance on Days 16, 17, 18, 19, 20, and 21, and exhibited few feces on Day 20. All animals exhibited body weight gain during the study.

Dermal Reactions to T-7280 at Induction and Initial Challenge. Individual dermal reactions for the test and control animals are presented in Table 6. Very faint to moderate erythema reactions were elicited from all 20 test animals to the undiluted test material during the induction phase of the study.

Very faint erythema reactions (maximum score of 0.5, 6 of the 20 test animals), faint erythema reactions (maximum score of 1.0, 9 of the 20 test animals), and moderate erythema reactions (maximum score of 2.0, 4 of the 20 test animals) were elicited following the challenge application of the 75% w/v mixture of T-7280 in 80% v/v ethanol in distilled water. None of the naive control animals reacted to the challenge application of the test material. Nineteen of the reactions in the test group to T-7280 exceeded the highest naive control reaction to T-7280 during the initial challenge phase of the study.

Dermal Reactions to Curing Agent 5974P at Rechallenge. Individual dermal reactions for the test and control animals at rechallenge are presented in Table 6. Very faint erythema reactions (score of 0.5) were elicited from 6 of the 20 test animals following the rechallenge application of the 10% w/v mixture of Curing Agent 5974P in distilled water. None of the additional naïve control animals reacted to the rechallenge application of the 10% w/v mixture of Curing Agent 5974P in distilled water.

Although six of the reactions in the test group to Curing Agent 5974P exceeded the highest naive control reaction to this same material during the rechallenge phase of the study, none of these reactions are significant enough to be considered definitive sensitization reactions.

Positive Control Report. A study report detailing the results of sensitization testing of α-hexylcinnamaldehyde (a known skin sensitizer) using the same sensitization method is presented in Appendix 2. This positive control study was conducted within 6 months of the conduct of this study.

CONCLUSION

Based on the results obtained in the initial challenge phase, T-7280 is considered to be a dermal sensitizer in guinea pigs when tested by the closed patch technique. The component material , Curing Agent 5974P, was not shown to cause definitive sensitization reactions in animals previously sensitized to T-7280.

SIGNATURE

Steven M. Glaza
Study Director
Toxicology

1-27-00
Date

REFERENCES

1. United States Environmental Protection Agency: Prevention, Pesticides and Toxic Substances: "OPPTS 870.2600 Skin Sensitization": *Health Effects Test Guidelines* (August 1998).

2. "Skin Sensitisation," *Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

3. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 1

Individual Body Weights and Dermal Reactions - Initial Irritation Screening Study – T-7280

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (% w/v in 80% v/v ethanol in distilled water)							
		25%		50%		75%		100%÷	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E20058	511	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E20066	483	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E20067	486	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E20069	532	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5

÷ Test material administered undiluted.

Table 2

Individual Body Weights and Dermal Reactions - Second Irritation Screening Study – T-7280

Animal Number	Initial Body Weight (g)	Dermal Reactions: Test Material Concentration (w/v % in 80% v/v Ethanol in Distilled Water)							
		25%		50%		75%		100%÷	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E20271	508	0.5	0.5	0.0	0.0	0.0	0.0	1.0	1.0
E20272	506	0.0	0.0	0.5pp	0.0pp	0.5pp	0.0pp	0.5pp	0.5pp
E20273	509	0.5pp	0.5pp	0.0	0.5	0.0	0.5	0.5	0.5
E20274	519	0.0	0.0	0.0	0.0	0.0	0.0	0.5pp	0.5pp

pp Pinpoint areas of subcutaneous hemorrhaging.

Comment: All animals appeared normal throughout this irritation screening study.

Table 3

Individual Body Weights and Dermal Reactions - Third Irritation Screening Study – Curing Agent 5974P

Animal Number	Initial Body Weight (g)	Dermal Reactions							
		Test Material Concentration (w/v % in Distilled Water)							
		10%		25%		50%		75%	
		Hour		Hour		Hour		Hour	
		24	48	24	48	24	48	24	48
E20536	403	0.5	0.5	1.0	1.0	1.0	1.0	2.0	2.0
E20545	532	0.0	0.0	0.5	0.5	0.5	0.5	1.0	1.0
E20547	490	0.0	0.0	0.5	0.0	1.0	1.0	2.0	2.0
E20548	422	0.0	0.0	0.0	0.0	0.5	0.5	1.0	1.0

Comment: All animals appeared normal throughout this irritation screening study.

Table 4

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
	Test Group	
E20278	435	780
E20279	465	831
E20280	488	854
E20281	468	882
E20282	454	778
E20283	467	786
E20284	451	712
E20285	425	777
E20286	451	816
E20287	435	855
E20288	442	820
E20289	463	844
E20290	414	695
E20291	456	811
E20292	454	833
E20293	454	870
E20294	423	731
E20295	446	845
E20296	450	755
E20297	409	773

Table 4(Continued)

Individual Body Weights (g) - Definitive Study

Animal Number	Predose	Terminal
Naive Control Group		
E20298	435	706
E20299	428	706
E20300	453	619
E20301	449	768
E20302	439	724
E20303	420	709
E20304	464	738
E20305	447	698
E20306	456	808
E20307	403	626
Additional Naive Control Group		
E20675	531	566
E20722	606	612
E20724	597	622
E20726	545	591
E20729	554	595

Table 5

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-14	15	16	17	18	19	20	21	22-42	43	44	45-56	57	58	59	60	61
E20278	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20279	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20280	Normal appearance	✓	-	-	-	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Appears dehydrated*	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Thin appearance	-	-	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-
	Few feces	-	-	-	-	-	-	✓	-	-	-	-	-	-	-	-	-	-
E20281	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20282	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20283	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20284	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20285	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20286	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20287	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20288	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20289	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20290	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20291	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20292	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20293	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20294	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20295	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20296	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E20297	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed
- Condition not evident
* Given water bottle

Table 5 (Continued)

Individual Clinical Signs - Definitive Study

Animal Number	Observation	Day 1-14	15	16	17	18	19	20	21	22-42	43	44	45-56	57	58	59	60	61
									Naive Control									
E20298	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20299	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20300	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20301	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20302	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20303	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20304	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20305	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20306	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
E20307	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	*	*	*	*	*	*	*	*
									Additional Naive Control									
E20675	Normal appearance	**	**	**	**	**	**	**	**	**	**	**	**	✓	✓	✓	✓	✓
E20722	Normal appearance	**	**	**	**	**	**	**	**	**	**	**	**	✓	✓	✓	✓	✓
E20724	Normal appearance	**	**	**	**	**	**	**	**	**	**	**	**	✓	✓	✓	✓	✓
E20726	Normal appearance	**	**	**	**	**	**	**	**	**	**	**	**	✓	✓	✓	✓	✓
E20729	Normal appearance	**	**	**	**	**	**	**	**	**	**	**	**	✓	✓	✓	✓	✓

✓ Condition existed
* Not applicable, animal terminated on Day 42
** Not applicable, animal added to study on Day 57

Table 6

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase[a]						Challenge Phase[b]		Rechallenge Phase[c]	
	Dose #1		Dose #2		Dose #3					
	Hour		Hour		Hour		Hour		Hour	
	24	48	24	48	24	48	24	48	24	48
Test Group										
E20278	1.0	1.0	1.0	1.0	0.5	1.0	1.0	0.5	0.5	0.5
E20279	1.0	1.0	1.0	0.5	1.0	2.0	1.0	0.5	0.0	0.0
E20280	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.5	0.5
E20281	1.0	1.0	1.0	0.5	1.0	2.0	1.0	1.0	0.0	0.0
E20282	1.0	2.0	2.0	2.0	1.0	1.0	2.0	1.0	0.5	0.0
E20283	0.5	0.5	0.5	0.5	1.0	1.0	0.0	0.0	0.0	0.0
E20284	2.0	2.0	1.0	1.0	0.5	0.5	0.5	0.0	0.0	0.0
E20285	1.0	1.0	1.0	1.0	0.5	0.5	1.0	0.5	0.0	0.0
E20286	1.0	1.0	1.0	1.0	0.5	0.5	1.0	0.5	0.0	0.0
E20287	1.0	1.0	1.0	1.0	0.5	1.0	1.0	0.5	0.5	0.5
E20288	0.5	1.0	0.5	0.5	1.0	1.0	1.0	1.0	0.5	0.5
E20289	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.5	0.0	0.0
E20290	1.0	1.0	1.0	1.0	1.0	2.0	1.0	2.0	0.0	0.0
E20291	1.0	0.5	0.5	0.5	1.0	1.0	0.5	0.0	0.0	0.0
E20292	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.0	0.0	0.0
E20293	1.0	1.0	1.0	1.0	1.0	2.0	2.0	2.0	0.0	0.0
E20294	1.0	0.5	1.0	0.5	0.5	1.0	1.0	1.0	0.0	0.0
E20295	0.5	0.5	0.5	0.5	1.0	1.0	0.5	0.0	0.0	0.0
E20296	0.5	0.5	1.0	1.0	0.5	1.0	1.0	0.5	0.0	0.0
E20297	0.5	0.5	1.0	1.0	0.5	1.0	1.0	2.0	0.5	0.5

a T-7280 applied undiluted.

b T-7280 applied as a 75% w/v mixture in 80% v/v ethanol in distilled water.

c Curing Agent 5974P applied as a 10% w/v mixture in distilled water.

Table 6 (continued)

Individual Dermal Reactions - Definitive Study

Animal Number	Induction Phase[a] Dose #1 Hour 24	Dose #1 Hour 48	Dose #2 Hour 24	Dose #2 Hour 48	Dose #3 Hour 24	Dose #3 Hour 48	Challenge Phase[b] Hour 24	Challenge Phase[b] Hour 48	Rechallenge Phase[c] Hour 24	Rechallenge Phase[c] Hour 48
Initial Naïve Control Group										
E20298	*	*	*	*	*	*	0.0	0.0	*	*
E20299	*	*	*	*	*	*	0.0	0.0	*	*
E20300	*	*	*	*	*	*	0.0	0.0	*	*
E20301	*	*	*	*	*	*	0.0	0.0	*	*
E20302	*	*	*	*	*	*	0.0	0.0	*	*
E20303	*	*	*	*	*	*	0.0	0.0	*	*
E20304	*	*	*	*	*	*	0.0	0.0	*	*
E20305	*	*	*	*	*	*	0.0	0.0	*	*
E20306	*	*	*	*	*	*	0.0	0.0	*	*
E20307	*	*	*	*	*	*	0.0	0.0	*	*
Additional Naïve Control Group										
E20675	*	*	*	*	*	*	*	*	0.0	0.0
E20722	*	*	*	*	*	*	*	*	0.0	0.0
E20724	*	*	*	*	*	*	*	*	0.0	0.0
E20726	*	*	*	*	*	*	*	*	0.0	0.0
E20729	*	*	*	*	*	*	*	*	0.0	0.0

* Animals not tested during this phase.

a T-7280 applied undiluted.

b T-7280 applied as a 75% w/v mixture in 80% v/v ethanol in distilled water.

c Curing Agent 5974P applied as a 10% w/v mixture in distilled water.

APPENDIX 1

Protocol
Protocol Amendment No. 1

Sample Submittal Form

This form is to be used when submitting samples for routine acute testing. Special testing needs can be easily arranged by contacting the Acute Studies Department (Madison) at (608) 241-7292

Covance Study No. 90703666

Enclose with samples and send to:

[X] Covance Laboratories
3301 Kinsman Boulevard
Madison, WI 53704

[ ] Covance Laboratories
9200 Leesburg Pik
Vienna, VA 2218

Submitted by: Ken Nakatani

Date Sample Sent: _____

Company: 3M

Number of Reports Required: 3

Full GLP Compliance: [ ] No [X] Yes → [ ] FDA (21 CFR 58) [ ] EPA (TSCA-40 CFR 792) [X] OECD

Sample Name: T-7280

Physical Description: Liquid with amine odor

Special Handling Precautions: Material is a skin corrosive

Test Material purity and stability information (including under test conditions) on file with Sponsor: [X] Yes [ ] No

Note: Test material/vehicle mixtures prepared at Covance will not be analyzed for concentration or homogeneity unless specifically requested by the Sponsor.

Sample Disposal: [X] Dispose of according to Covance SOPs
[ ] Return to Sponsor at the following address
→ Incinerate in an industrial or commercial incinerator.

Sample Storage Requirements:
[X] Room temperature
[ ] Refrigerated
[X] Other Cool Place

Tests

Acute Oral Toxicity in Rats
[ ] TP2069 EPA/OECD screen: 5M-5F at 2,000 mg/kg
[ ] Conduct defined LD_{50} study if death occurs at 2,000 mg/kg
[ ] Optional -- initial dose level at 5,000 mg/kg

Special instructions: _____

Acute Dermal Toxicity in Rabbits
[ ] TP2070 EPA/OECD screen: 5M-5F at 2,000 mg/kg
[ ] Conduct defined LD_{50} study if death occurs at 2,000 mg/kg

Special instructions: _____

Dermal Absorption Studies
[ ] Single-dose intravenous pharmacokinetic
[ ] 5-Daily dose dermal absorption/toxicity

Special instructions: _____

Primary Dermal Irritation
[ ] TP3208 FHSA: 6 rabbits – 1 abraded, 1 intact site/rabbit
[ ] TP2071 EPA/OECD: 3 rabbits – 1 intact site/rabbit
[ ] TP4206 DOT/OECD corrosivity: 3 rabbits – 3 exposure sites/rabbit

Special instructions: _____

Primary Eye Irritation
[ ] TP2072 EPA/OECD: 3 rabbits unwashed

Special instructions: _____

Guinea Pig Sensitization
[ ] TP2017 Maximization - Screening Design (23 animals)
[X] TP6164 EPA/OECD/EC Maximization (38 animals)
[X] TP2008 Buehler sensitization (34 animals)
[ ] Optional – conduct with 10 animals in test group (24 to
[ ] TP2008 B Buehler sensitization (9 induction apps., 20 anim

Special instructions: Retain animals for possible rechallenge w/ compound. Material (Curing Agent 5974P). Follow protocols used for T-7262. SRS 9-10-99

For Covance Use Only

Protocol Issue Date: 9-10-99

Study Director: _____

① Per the Sponsor we are to conduct the TP2008 study design, using an appropriate vehicle. Control data may be found for the TP6164 study design. SRS 9-10-99

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL TP2008

Study Title:

Dermal Sensitization Study in Guinea Pigs-
Closed Patch Technique
(EPA/OECD Guidelines)

Date:

December 22, 1998

Laboratory Project Identification:

Covance 90703666.

Study

Dermal Sensitization Study in Guinea Pigs - Closed Patch Technique (EPA/OECD Guidelines)

Purpose

To assess the delayed contact hypersensitivity potential of a test material in guinea pigs.

Sponsor

3M
Toxicology Services
St. Paul, MN

Sponsor's Representative

John L. Butenhoff, PhD
3M
Toxicology Services
3M Center, Bldg. 220-2E-02
St. Paul, MN 55144
Telephone: 651.733.1962

3M Study Monitor

(See Sample Submittal Form)

Study Director

☒ Steven M. Glaza
Covance Laboratories Inc.
Madison, WI.
Telephone 608.241.7292
Facsimile 608.242.7936

Testing Facility

☒ Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

☐ Covance Laboratories Inc.
9200 Leesburg Turnpike
Vienna, VA 22182-1699

Proposed Study Timetable

Experimental Start Date	Week of 9-13-99
Experimental Termination Date	Week of 10-18-99
Final Report Date	Week of 11-29-99

Regulatory Compliance

This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards, with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility and stability will not be conducted:

[ ] Conduct as a Nonregulated Study
[ ] 21 CFR 58 (FDA)
[ ] 40 CFR 160 (EPA-FIFRA)
[ ] 40 CFR 792 (EPA-TSCA)
[X] C(97)186/Final (OECD)

Animal Care and Use Statement

All procedures in this protocol are in compliance with the Animal Welfare Act and/or the Guide for the Care and Use of Laboratory Animals and the Office of Protection from Research Risks. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.

Quality Assurance

For regulated studies, the protocol, study conduct, and the final report will be audited by Covance's Quality Assurance Unit.

Test Material

Identification

(See Sample Submittal Form)

Physical Description

(See Sample Submittal Form)

Purity and Stability
The Sponsor assumes responsibility for purity and stability determinations (including under test conditions).

Storage Conditions
(See Sample Submittal Form)

Reserve Samples
Reserve samples of each batch/lot of the test material will be taken and stored at Covance in a freezer set to maintain a temperature of -20°C ±10°. The reserve samples will be maintained as outlined in the Record Retention section.

Disposition
Any unused test material will be discarded after issuance of the final report, unless otherwise directed by the Sponsor.

Animals

Species
Guinea Pig

Strain/Source
Crl:(HA)BR, Charles River Laboratories Inc.

Age at Initiation of Treatment
Young adult

Weight at Initiation of Treatment
350 to 550 g. Animals falling outside this range can be used at the discretion of the Study Director.

Number and Sex
34 of any sex

☐ Optional procedure: 24 of any sex.

Identification
Individual numbered ear tag

Husbandry

Housing
Individually, in suspended, screen-bottom stainless steel cages

Diet
Certified Guinea Pig Diet #5026 (PMI Nutrition International), *ad libitum*. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants.

Water
Ad libitum from an automatic system. Samples of the water are periodically analyzed for specified microorganisms and environmental contaminants.

Contaminants
There are no known contaminants in the diet or water at levels that would be expected to interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain a temperature of 18° to 26°C, a relative humidity of 50% ±20%, and a 12-hour light/12-hour dark cycle.

Acclimation
At least 5 days

Selection of Test Animals
Based on health and body weight according to Covance SOPs. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

Treatment Procedures

Experimental Design

Thirty-four acclimated animals weighing between 350 and 550 g will be placed into an irritation screening group of four guinea pigs, a naive control group of 10 guinea pigs, and a test group of 20 guinea pigs.

☐ Optional procedure: the test group will consist of 10 animals.

Irritation Screen

An irritation screening study of four guinea pigs will be conducted. The dosing procedure will be in the same manner as the definitive study. Four application sites will be selected on each animal. Each site will be treated with 0.4 mL of one of the following concentrations 25%, 50%, 75% w/v in an appropriate vehicle or 100% (undiluted). For solid test materials, a 0.4-g dose of the test material (just moistened with sterile water) will be used where the undiluted (100%) test material is specified. The mild to moderately irritating concentration will be used in the induction phase of the definitive study. The highest nonirritating concentration will be used for the challenge application.

If a nonirritating concentration is not found, additional testing using new animals will be employed.

All test mixtures used in the irritation screen or definitive phases of the study will be stored at room temperature until administered.

Dose Administration (Definitive Study)

Before each application the back of each animal will be clipped free of hair with an electric clipper. The test material will be applied to one area on each test animal by placing 0.4 mL (or a 0.4-g dose) of the test material on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch will be covered with dental dam and secured

with an overwrap of Elastoplast® tape. The dressing will remain in place for approximately 6 hours, and the residual material will be removed using water or an appropriate substance and disposable paper towels. The test animals will receive one application each week for 3 weeks for a total of three applications. Each application will be made on the same site during the induction phase unless moderate to severe irritation is observed following the first or second induction application. The induction site may then be moved slightly posterior to the initial induction site.

Two weeks after the administration of the third induction dose, a challenge dose, at a volume of 0.4 mL (or 0.4-g dose) of the highest nonirritating concentration, will be administered to the test group along the dorsal anterior right quadrant and in the same manner as during the induction phase of the study. At this time, the 10 naive control animals will also be treated with a challenge application of the test material.

The study will terminate after the 48 hour challenge application scoring unless equivocal responses are observed. If equivocal responses are observed in the initial challenge application, the test and naive control animals may be given a second challenge as determined by the study director. The second challenge will occur 1 to 2 weeks after the initial challenge application.

Reason for Route of Administration
Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observation of Animals

Clinical Observations
Daily clinical signs will be recorded.

Body Weights
Before test material administration (Irritation Screen and Definitive Study animals) and at the termination of the experimental phase (Definitive Study animals only).

Reading of Dermal Reactions

The respective application sites will be examined and scored for dermal irritation, according to the scoring scale in Attachment 1, at approximately 24 and 48 hours after the irritation screen, induction, and challenge applications.

On the day of the 24-hour examination following the irritation screen and challenge applications, the respective test sites will be depilated by applying Neet® for approximately 10-20 minutes, then washed off with water. The 24-hour examination must take place at least 2 hours after removal of the depilatory.

Pathology

Any animals dying during the study, or sacrificed in a moribund condition, will be subjected to an abbreviated macroscopic necropsy examination and all abnormalities will be recorded. After necropsy, the animals will be discarded and no tissues will be saved. At termination of the respective experimental phase, surviving animals will be sacrificed and discarded.

Statistical Evaluation

No statistical evaluations are required.

Report

A final report including the items listed below will be submitted.

Description of the test material
Description of the test system
Procedures
Dates of experimental initiation and termination
Summary table showing the induction and challenge responses
Any special observations that were recorded
Determination of sensitization based upon reactions to the challenge dose. Grades of 1 or greater in the test animals indicate evidence of sensitization, provided grades of less than one are seen in the naive control animals. If grades of one or greater are noted in the naive control animals, then the reactions of test animals exceeding the most severe naive control reactions are considered sensitization reactions.

A positive control report from periodic validation of the test method will be presented as an appendix in the final report.
Macroscopic pathology findings (if applicable)

Record Retention

All raw data, documentation, records, test material reserve samples, protocol, protocol amendments (if applicable), and the final report generated as a result of this study will be archived in the storage facility of Covance for a period of one year following signing of the final report. One year after signing of the final report, all of the aforementioned materials may be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, for which Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged. All raw data stored on magnetic media will be retained by Covance.

The following supporting records will be retained at Covance but will not be archived with the study data:

Animal receipt/acclimation records
Water analysis records
Animal room maintenance and environmental records
Refrigerator, freezer and room temperature records
Positive control data
Feed analysis records
Instrument calibration and maintenance records

PROTOCOL APPROVAL

John L. Butenhoff, PhD
Sponsor's Representative
3M

12/30/98
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

12-22-98
Date

Attachment 1

Buehler Sensitization Scoring Scale

No reaction	0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Buehler, E.V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests, "Current Concepts in Cutaneous Toxicity, p. 28 (1980)

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Covance 90703666

Dermal Sensitization Study in Guinea Pigs-Closed Patch Technique
(EPA/OECD Guidelines)

Sponsor:	3M, St. Paul, MN
Sponsor's Representative:	John L. Butenhoff, PhD
3M Study Monitor	Ken Nakatani, MS, DABT
Testing Facility:	Covance Laboratories Inc., Madison, WI
Study Director:	Steven M. Glaza

This amendment modifies the following portions of the protocol:

Effective October 26, 1999

1. **Page 6, Irritation Screen.** Based on the results of the challenge application, a rechallenge application may be conducted. To identify the actual procedure to be used for the additional irritation screening studies to determine the concentrations to be used for the rechallenge application (if necessary), add the following as the fourth paragraph of this section:

   A third irritation screening using a test material component identified as Curing Agent 5974P will be conducted using four new guinea pigs. The dosing procedure will be in the same manner as the previous irritation screening study applications. For the component test material (Curing Agent 5974P) treat four guinea pigs with the following test material concentrations: 10%, 25%, 50%, and 75% w/v in distilled water. There will be four sites on each animal (one site per concentration).

Covance 90703666

Effective November 16, 1999

2. **Page 7, Dose Administration (Definitive Study).** Based on the results of the initial challenge application, a rechallenge application will be conducted. To identify the actual procedure to be used for the rechallenge application, add the following as the fourth paragraph of this section:

   A rechallenge application phase will be conducted in the same manner as the initial challenge application. Treat the test group and five additional naive control animals (weighing from 400 to 700 g) with the following test material concentration: 10% w/v Curing Agent 5974P in distilled water. A naive site will be used on each animal.

PROTOCOL AMENDMENT APPROVAL

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

12-20-99
Date

(90703666.am1)

APPENDIX 2

Positive Control Report

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

Covance Laboratories Inc.
Madison, WI

FINAL REPORT

Study Title:

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde in Guinea Pigs - Closed Patch Technique

Data Requirement:

OPPTS 870.2600

Author:

Steven M. Glaza

Study Completion Date:

August 4, 1999

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Laboratory Project Identification:

Covance 90502240

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of the United States Environmental Protection Agency Federal Insecticide, Fungicide, and Rodenticide Act §10 (d) (1) (A), (B), or (C).

Covance Laboratories Inc.
Company

Steven M. Glaza
Company Agent

8-4-99
Date

Study Director
Title

Steven M Glaza
Signature

COMPLIANCE STATEMENT

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

This study was conducted in accordance with the following Good Laboratory Practice Regulations/Standards with the exception that analysis of the test material mixtures for concentration, homogeneity/solubility, and stability was not conducted:

United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR 58

United States Environmental Protection Agency FIFRA: Good Laboratory Practice Standards, 40 CFR 160

United States Environmental Protection Agency TSCA: Good Laboratory Practice Standards, 40 CFR 792

Organisation for Economic Cooperation and Development Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17

Japanese Ministry of Agriculture, Forestry and Fisheries, 59 NohSan Notification No. 3850

Japanese Ministry of Health and Welfare, Ordinance 21

Management
Covance Laboratories Inc.

8 4 99
Date

Steven M. Glaza
Study Director
Acute Studies
Covance Laboratories Inc.

8-4-99
Date

QUALITY ASSURANCE STATEMENT

This report has been reviewed by the Quality Assurance Unit of Covance Laboratories Inc., in accordance with the Food and Drug Administration (FDA) Good Laboratory Practice Regulations, 21 CFR 58, the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17, the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 160, the Environmental Protection Agency (EPA) Good Laboratory Practice Standards, 40 CFR 792, the Japanese Ministry of Agriculture, Forestry, and Fisheries (MAFF) Good Laboratory Practice Standards, 59 NohSan No. 3850, and the Japanese Ministry of Health and Welfare (MOHW), Good Laboratory Practice Standards, Ordinance 21. The following inspections were conducted and findings reported to the study director and study director management. Written status reports of inspections and findings are issued to Covance management according to standard operating procedures.

Inspection Dates			Date Reported to Study Director and Study Director Management
From	To	Phase	
05/20/99	05/20/99	Protocol Review	05/20/99
06/02/99	06/02/99	Animal Observation	06/02/99
08/02/99	08/02/99	Data Review	08/02/99
08/02/99	08/02/99	Report Review	08/02/99

Susan K. Gustafson
Representative, Quality Assurance Unit

8-4-99
Date

STUDY IDENTIFICATION

Dermal Sensitization Study of Alpha-Hexylcinnamaldehyde
in Guinea Pigs - Closed Patch Technique

Test Material	Alpha-Hexylcinnamaldehyde
Sponsor	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Director	Steven M. Głaza Covance Laboratories Inc. Telephone No.: 608.241.7292 Facsimile No.: 608.242.7936
Test Facility	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Study Timetable	
Study Initiation Date	May 21, 1999
Experimental Start Date	May 27, 1999
Experimental Termination Date	June 26, 1999

KEY PERSONNEL

Acute Studies

Steven M. Glaza
Study Director

Steven R. Sorenson
Study Coordinator

Rose M. Bridge
Administrative Supervisor

Toxicology Operations

Jeffrey B. Hicks
In-life Supervisor

Quality Assurance

Nancy M. Centanni
Manager

Laboratory Animal Medicine

Donna J. Clemons, DVM
Diplomate, ACLAM
Associate Director

CONTENTS

SUMMARY

The delayed contact hypersensitivity potential of Alpha-Hexylcinnamaldehyde (HCA) was evaluated in albino guinea pigs. The test material was administered as a 2.5% w/v mixture in ethanol to each animal in the test group during the three-application induction phase of the study. During the challenge phase of the study, HCA was administered to each animal in the test and naive/irritation control groups at concentrations of 1.0, 2.5, and 5.0% w/v in acetone (three sites per animal).

Very faint to faint erythema reactions (scores of 0.5 to 1.0) were observed in seven of the 10 test animals while no erythema reactions were seen in any of the five naive/irritation control animals when HCA was administered as a 1.0% w/v mixture in acetone at challenge. Very faint to faint erythema reactions (scores of 0.5 to 1.0) were observed in eight of the 10 test animals while no erythema reactions were seen in any of the five naive/irritation control animals when HCA was administered as a 2.5% w/v mixture in acetone at challenge. Very faint to moderate erythema reactions (scores of 0.5 to 2.0) were observed in all 10 test animals while very faint erythema reactions (score of 0.5) were seen in three of the five naive/irritation control animals when HCA was administered as a 5.0% w/v mixture in acetone at challenge. In the test group at challenge, two reactions to the 1.0% w/v mixture, two reactions to the 2.5% w/v mixture, and eight reactions to the 5.0% w/v mixture are considered to be sensitization reactions (scores of 1.0 or 2.0). Based on these results, HCA was shown to be a dermal sensitizer in guinea pigs.

OBJECTIVE

The objective of this study was to assess the delayed contact hypersensitivity potential of HCA in guinea pigs.[1,2,3,4]

All procedures used in this study were in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office for Protection from Research Risks. In the opinion of the Sponsor and study director, the study did not unnecessarily duplicate any previous work. The dose levels, method, frequency, and duration of administration utilized in this study were chosen based on the requirements of

the regulatory test guidelines. All procedural times presented in this report fall within the acceptable ranges as specified in Covance standard operating procedure (SOP).

TEST MATERIAL

Identification

The test material was identified as alpha-hexylcinnamaldehyde [HCA (Aldrich Chemical Company, Lot No. 10021HF)] and described as a clear, yellow liquid.

Purity and Stability

The purity and stability of this reagent grade material were the responsibility of Covance and were deemed adequate for the conduct of this study. The certificate of analysis will be retained in the study file.

Storage and Retention

The test material was stored at room temperature. A reserve sample of the test material was taken and will be retained for 10 years in a freezer set to maintain a temperature of -10 to -30°C in accordance with Covance SOP. Any unused test material will be retained for future tests until the indicated expiration date is reached.

TEST SYSTEM

Test Animal

Young adult albino guinea pigs of the Crl:(HA)BR strain were procured from Charles River Laboratories, Inc., Kingston, New York, on May 18, 1999.

Housing

After receipt, the animals were acclimated for a period of at least 5 days. During acclimation and throughout the study, the animals were individually housed in suspended, stainless steel cages. Environmental controls for the animal room were set to maintain a temperature of 18 to 26°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour

dark cycle. In cases where variations from these conditions existed, they were documented and considered to have had no adverse effect on the study outcome.

Animal Diet

The animals were provided continuous access to certified guinea pig diet (#5026, Purina Mills, Inc.) and water. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Samples of the water are periodically analyzed. There were no known contaminants in the diet or water at levels that would be expected to interfere with or affect the results of the study.

Group Assignments

Fifteen healthy, acclimated male albino guinea pigs, weighing from 362 to 412 g and approximately 6 weeks of age, were divided into two groups consisting of a test group of 10 animals and a naive/irritation control group of five animals. The animals were identified by animal number and corresponding ear tag throughout the study.

Justification for Species Selection

Historically, the albino guinea pig has been the animal of choice for skin sensitization studies.

PROCEDURES

Induction Phase

On each day of test material application, the hair was removed from the back of each animal in the test group with electric clippers. The test material was applied to each animal in the test group by placing 0.3 mL of a 2.5% w/v mixture of HCA in ethanol on an adhesive patch (Hill Top Chamber®, 25-mm diameter) and placing the patch on the induction site along the dorsal anterior left quadrant. The patch was covered with dental dam and overwrapped with Elastoplast® tape. The dressing remained in place for a period of 6 hours after which it was removed. Any residual material was then removed from the application sites using water and disposable paper towels. The animals in the test group received one application per week for 3 weeks for a total of three applications, with the

third induction application for the 10 animals in the test group being applied slightly posterior to the previous induction site on these animals. The naive/irritation control animals were not treated during this phase of the study. All test material mixtures used in the study were stored at room temperature until administered.

Challenge Phase

Two weeks following the administration of the third induction dose, a challenge dose of HCA at concentrations of 1.0, 2.5, and 5.0% w/v in acetone was administered on three respective challenge sites (0.3 mL volume/site) along the right side of the test group animals in the same manner as during the induction phase of the study. At this time the five naive/irritation control animals (previously untreated) were also treated in the same manner with a challenge application of the same three test material concentrations.

Reason for Route of Administration

Historically, the dermal route has been the route of choice for determining delayed contact hypersensitization.

Observations

On the day of the 24-hour examination following the challenge application, the application sites of the respective animals were depilated by applying Neet® depilatory. After approximately 10 to 20 minutes, the depilatory was washed from the application sites. The 24-hour examination occurred at least 2 hours after removal of the depilatory.

The respective application sites were examined and scored for dermal reactions according to the Buehler[5] scoring scale at 24 and 48 hours following the challenge application.

Clinical observations were conducted daily throughout the study. Body weights were determined before the initial test material administration and at termination of the in-life phase.

Termination

At termination of the in-life phase, all animals were euthanized and discarded.

Evaluation of Challenge Responses

Determination of sensitization was based on the dermal reactions to the challenge dose. Grades of 1.0 or greater in the test animals indicate evidence of sensitization, provided grades of less than 1.0 are seen in the naive/irritation control animals.

Statistical Evaluation

No statistical evaluations were required by the protocol.

Location of Raw Data, Records, and Final Report

The raw data, records, and an original signed copy of the final report will be retained for 10 years in the archives of Covance in accordance with Covance SOP.

RESULTS/DISCUSSION

Clinical Observations and Body Weights

Individual body weights are in Table 1 and individual clinical signs are in Table 2. All animals in both groups appeared normal and exhibited body weight gain throughout the study.

Dermal Reactions to HCA at Challenge

Individual dermal reactions at challenge for the test and naive/irritation control animals are presented in Table 3.

1.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint (score of 0.5 in five animals) to faint (score of 1.0 in two animals) erythema reactions at the challenge sites treated with the 1.0% w/v mixture of HCA in acetone at challenge. None of the five animals in the naive/irritation control group exhibited any erythema reaction at the sites treated with the 1.0% w/v mixture. Two of the reactions in the test group (score of 1.0) are considered to be sensitization reactions.

2.5% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint (score of 0.5 in six animals) to faint (score of 1.0 in two animals) erythema reactions at the challenge sites treated with the 2.5% w/v mixture of HCA in acetone. None of the animals in the naive/irritation control group exhibited any erythema reaction at the sites treated with the same 2.5% w/v mixture. Two of the challenge reactions in the test group (score of 1.0) are considered to be sensitization reactions.

5.0% w/v Mixture of HCA in Acetone

Animals in the test group exhibited very faint to moderate erythema reactions (score of 0.5 in two animals, 1.0 in five animals, and 2.0 in three animals) at the challenge sites treated with the 5.0% w/v mixture of HCA in acetone. Three of the animals in the naive/irritation control group exhibited very faint erythema reactions (score of 0.5) at three of the five sites treated with the same 5.0% w/v mixture. Eight of the reactions in the test group (scores of 1.0 or 2.0) are considered to be sensitization reactions.

CONCLUSION

Based on the results obtained, this test material, HCA was shown to be a dermal sensitizer in guinea pigs when tested by the closed patch technique.

SIGNATURE

Steven M. Glaza
Study Director
Acute Studies

8-4-99
Date

REFERENCES

1. United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances; "OPPTS 870.2600 Skin Sensitization"; *Health Effects Test Guidelines* (August 1998).

2. "Dermal Sensitization Study," *Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration*, Ministry of Agriculture Forestry and Fisheries, 59 NohSan No. 4200, January 28, 1985.

3. "Skin Sensitization Studies," *1990 Guidelines for Toxicity Studies of Drugs Manual*, First Edition, Ch. 7, pp. 75-80, YAKUJI NIPPO, LTD. (1991).

4. "Skin Sensitisation," *Organisation for Economic Cooperation and Development Guidelines for Testing of Chemicals*, Section 4, Health Effects, Number 406, Paris Cedex (July 17, 1992).

5. Buehler, E. V. and Ritz, H. L., "Planning, Conduct, and Interpretation of Guinea Pig Sensitization Patch Tests," *Current Concepts in Cutaneous Toxicity*, p. 28 (1980).

Table 1

Individual Body Weights (g)

Animal Number	Predose	Terminal
	Test Group	
E18837	383	586
E18838	404	627
E18839	362	556
E18840	412	661
E18841	399	594
E18842	368	548
E18843	403	604
E18844	404	696
E18845	402	579
E18846	384	536
	Naive/Irritation Control Group	
E18847	399	582
E18848	377	517
E18849	408	594
E18850	386	552
E18851	412	638

Table 2

Individual Clinical Signs

Animal Number	Observation	Day																
		1-15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Test Group																		
E18837	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18838	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18839	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18840	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18841	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18842	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18843	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18844	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18845	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18846	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Naive/Irritation Control Group																		
E18847	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18848	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18849	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18850	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
E18851	Normal appearance	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ Condition existed.

Buehler Sensitization Scoring Scale

No reaction	0.0
Very faint erythema, usually nonconfluent	0.5
Faint erythema, usually confluent	1.0
Moderate erythema	2.0
Strong erythema, with or without edema	3.0

Table 3

Individual Dermal Reactions at Challenge

Animal Number	Test Material Concentration (% w/v in Acetone)					
	1.0%		2.5%		5.0%	
	Hour		Hour		Hour	
	24	48	24	48	24	48
Test Group						
E18837	0.5	0.5	0.5	0.5	1.0	1.0
E18838	0.5	0.0	0.5	0.5	1.0	1.0
E18839	0.0	0.0	0.0	0.0	0.5	0.5
E18840	0.5	0.5	0.5	0.5	1.0	1.0
E18841	1.0	1.0	1.0	1.0	2.0	2.0
E18842	1.0	0.5	1.0	1.0	2.0	2.0
E18843	0.5	0.0	0.5	0.0	1.0	0.5
E18844	0.0	0.0	0.0	0.0	0.5	0.5
E18845	0.0	0.0	0.5	0.5	1.0	2.0
E18846	0.5	0.5	0.5	0.5	1.0	0.5
Naive/Irritation Control Group						
E18847	0.0	0.0	0.0	0.0	0.0	0.0
E18848	0.0	0.0	0.0	0.0	0.5	0.0
E18849	0.0	0.0	0.0	0.0	0.5	0.0
E18850	0.0	0.0	0.0	0.0	0.0	0.5
E18851	0.0	0.0	0.0	0.0	0.0	0.0